

**Center for Veterinary Biologics
and
National Veterinary Services Laboratories
Testing Protocol**

**Supplemental Assay Method for the Manual Determination
of Formaldehyde in Veterinary Biologics (Schiff Test)**

Date: May 5, 1997

Supersedes: TCSAM0510.01

Number: TCSAM0510.02

Contact Person: Jan DeJong, 515-239-8511
email: jdejong@aphis.usda.gov

Approvals:

P. Frank Ross, Head
Toxicology and Chemistry Section

Date:_____

Arthur J. Davis, Chief
Pathobiology Laboratory

Date:_____

Richard E. Hill, Quality Assurance Manager

Date:_____

_/_s/ R. L. Levings
Randall L. Levings, Director
Center for Veterinary Biologics-Laboratory

Date: _5/21/97_

United States Department of Agriculture
Animal and Plant Health Inspection Service
P. O. Box 844
Ames, IA 50010

Mention of trademark or proprietary product does not constitute a guarantee or warranty of the product by USDA and does not imply its approval to the exclusion of other products that may be suitable.

Supplemental Assay Method for the Manual Determination of Formaldehyde
in Veterinary Biologics (Schiff Test)

Table of Contents

1. Introduction
2. Materials
 - 2.1 Equipment/instrumentation
 - 2.2 Reagents/supplies
3. Preparation for the test
 - 3.1 Personnel qualifications/training
 - 3.2 Preparation of equipment/instrumentation
 - 3.3 Preparation of reagents/control procedures
 - 3.4 Preparation of the sample
4. Performance of the test
5. Interpretation of the test results
6. Report of the test results
7. References
8. Appendices
 - 8.1 NVSL Media 30036
 - 8.2 NVSL Media 30019
 - 8.3 NVSL Media 30192

Supplemental Assay Method for the Manual Determination of Formaldehyde
in Veterinary Biologics (Schiff Test)

1. Introduction

This laboratory procedure may be used for the colorimetric determination of free formaldehyde in killed viral and bacterial products. The test is based on the classical Schiff's reaction for determining aldehydes.

2. Materials

2.1 Equipment/instrumentation--Brand names are provided for reference only, equivalent equipment or materials may be used.

2.1.1 Spectrophotometer or colorimeter with cuvettes (Bausch and Lomb Spectronic 70)

2.1.2 Centrifuge capable of 800-1000 x G.

2.1.3 Routine laboratory glassware and supplies, including pipets, screw cap test tubes, conical screw cap centrifuge tubes, test tube racks, class A volumetric flasks, laboratory toweling or wipes, laboratory timer, beakers, pipetor bulb (repipetors are optional), graph paper, and adjustable ship curve

2.2 Reagents/supplies--All chemicals reagent grade unless specified. Store at room temperature, unless otherwise specified. All chemicals and solutions good for 1 year, unless specified.

2.2.1 Dilute hydrochloric acid, 2.5% (**NVSL Media 30036, specify formula and volume, Appendix 8.1**)

2.2.2 Formaldehyde Solution, 37% (Formalin)--Check formaldehyde content of reagent by USP method (**Reference 7.2**). Prepare 1% standard formalin solution by diluting 1 ml of 37% formaldehyde to 100 ml with water (use 100 ml volumetric flask).

2.2.3 Modified Schiff Reagent, (**NVSL Media 30019, Appendix 8.2**) This reagent remains satisfactory for use for several weeks, as long as it retains a strong odor of sulfur dioxide.

2.2.4 Water--Use deionized or distilled water or water of equivalent purity.

Supplemental Assay Method for the Manual Determination of Formaldehyde
in Veterinary Biologics (Schiff Test)

2.2.5 Emulsified samples (oil and water emulsion) require that the emulsion be broken before the test is run. Chemicals needed for this step:

- 1). Sodium chloride, 10%--(NVSL Media 30192, specify formula and volume, Appendix 8.3)
- 2). Chloroform

3. Preparation for the test

3.1 Personnel training--No specific training is required. Individual should have working knowledge of laboratory equipment listed in **Section 2**.

3.2 Equipment--Turn on spectrophotometer to allow the instrument to "warm up" for at least 30 minutes.

3.3 Reagents/controls--Prepare a 1% formalin solution by diluting 1 ml of 37% formaldehyde with water to 100 ml in volumetric flask. Use this solution as a control, diluting it as the standard solution and testing it at 2 levels (run 0.2% and 0.4% or levels that bracket the samples).

3.4 Sample--Reference the current version of TCSOP0001 for sample submission.

Critical control point: Dilute the formalin standards and all samples to a constant ratio so that standards and samples are treated the same. The colorimetric reaction is very dependent upon time and temperature. The reaction is carried out at room temperature, consequently, it may be necessary to adjust the dilution to fit the conditions in each laboratory.

3.4.1 Dilute aqueous samples 1+3 (1+2 to 1+5) with water. Make duplicate dilutions.

3.4.2 Emulsified samples require that the emulsion be broken by adding 2 ml of sample to 4 ml of 10% NaCl and 6 ml of chloroform. This mixture is shaken for 30 seconds and then centrifuged for 5 minutes at 800-1000 x G. The clear upper layer is used in step 4.1.3. A separate standard curve should be prepared for these products in which the standards are treated the same as the emulsified samples.

Supplemental Assay Method for the Manual Determination of Formaldehyde
in Veterinary Biologics (Schiff Test)

4. Performance of the test

4.1 Samples

4.1.1 For each sample dilution, pipet 10 ml of 2.5% HCl into a tube and mark it (sample No._____).

4.1.2 Also for each sample dilution, pipet 12 ml of 2.5% HCl into a tube and mark it (sample blank No._____).

4.1.3 Place 1 ml of diluted sample into each of the tubes (**4.1.1 and 4.1.2**) and mix. Remember to make dilutions.

4.2 Standards

4.2.1 Pipet 2.5% HCl into a tube and mark the tube (standard blank). Use this tube to set the instrument at zero O.D.

4.2.2 Prepare tubes containing the 2.5% HCl and the diluted standard as described in the following table. Mark the tubes indicating the appropriate concentration of the standard.

Formalin equivalent(%)	0.1	0.2	0.3	0.4	0.5	0.6	0.7
ml of 2.5% HCl	10.9	10.8	10.7	10.6	10.5	10.4	10.3
ml of standard	0.1	0.2	0.3	0.4	0.5	0.6	0.7

4.2.3 Mix by gently inverting the tube.

4.3 In sequence, add 2 ml of Schiff Reagent to each tube marked sample or standard at 1 minute intervals. Do not add Schiff Reagent to any "blank" tubes. All tubes, at this point, should contain 13 ml.

4.4 Mix the tubes well but keep them in the proper sequence (mix immediately after the reagent is added).

4.5 Allow the color to develop for exactly 30 minutes from the time the first Schiff Reagent was added. Read O.D. at 570 nm and record the O.D. for each tube in sequence at 1 minute intervals.

4.6 Read and record the O.D. of each sample blank.

Supplemental Assay Method for the Manual Determination of Formaldehyde
in Veterinary Biologics (Schiff Test)

5. Interpretation of the test results

5.1 Draw a curve by plotting the O.D. of the standards versus the percent formalin.

5.2 Calculations

5.2.1 Subtract the O.D. of the sample blank from the O.D. of the sample. Record this difference.

5.2.2 Determine the percent formalin in the sample by comparing the net O.D. of the sample to the standard curve.

5.3 Retest

5.3.1 If the O.D. of the sample reads outside the end points of the standard curve, redilute the sample and retest (multiply results from the curve by this dilution factor).

5.3.2 If the color developed is extremely dark, redilute and retest. (If the color is faint, test at a lower dilution or check reagent, reagent may need to be replaced).

5.3.3 If the controls vary more than 10% from the expected value, make new standard and control solutions, redilute the sample, and retest.

6. Report of test results

6.1 Test results are reported following the current version of TCSOP0001.

7. References

7.1 Reference is made to the long term use of this procedure by the Toxicology and Chemistry Section.

7.2 U.S. Pharmacopia/National Formulary, current issue

7.3 This protocol is a revision of SAM 510, February 1, 1971, to the format presented here. Version .02 was generated to represent changes resulting from the creation of the Center for Veterinary Biologics. No technical changes from the previous version are represented.

Supplemental Assay Method for the Manual Determination of Formaldehyde
in Veterinary Biologics (Schiff Test)

Appendix 8.1 NVSL media 30036

Author's Comment: Media 30036 is generic and open-ended for HCl solutions. The requestor must request volume needed as well as the formulation. In the case of this protocol, specifics are:

Prepare 1 liter by slowly adding 25 ml concentrated HCl to approximately 500 ml water in a 1 liter volumetric flask. Fill to volume.

Appendix 8.2 NVSL media 30019

(Retyped here to fit the space)

SCHIFFS REAGENT, MODIFIED (STERILITY)

DISSOLVE 0.05g OF BASIC FUCHSIN IN 90ML OF DISTILLED WATER. THEN ADD 1g OF SODIUM SULFITE, MIX UNTIL COMPLETELY DISSOLVED AND THEN ADD 1ML OF CONCENTRATED HCL AND DILUTE TO ONE LITER WITH WATER. KEEP SOLUTION IN WELL-STOPPERED AMBER BOTTLE.

Appendix 8.3 NVSL media 30192

Author's Comment: Media 30192 is generic and open-ended for NaCl solutions. The requestor must request volume needed as well as the formulation. In the case of this protocol, specifics are:

Prepare 100 ml by adding 10 g NaCl to approximately 50 ml water in a 100 ml volumetric flask. Dissolve and fill to volume.